T-Cell Receptor-γ/δ Bearing Lymphocytes in Normal and Inflammatory Human Skin

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Murine dendritic epidermal T cells (DETC) were recently reported to express T-cell receptor (TCR)-γ/δ chains. In a search for the human equivalent of these cells, we tested normal and lesional skin with MoAb which react with the TCR-γ/δ heterodimer. We performed indirect immunofluorescence (IF) on epidermal sheets, and alkaline-phosphatase–anti-alkaline-phosphatase complex (APAAP) on epidermal cell smears. Frozen skin sections from normal skin and various cutaneous lymphocyte infiltrates were also studied. A few CD3+ T lymphocytes were consistently found in normal epidermis. Most of these cells appeared to be TCR-α/β+, and some CD4+ or CD8+. On epidermal sheets and cell smears, only a very small TCR-γ/δ+ cell population was visualized (less than 0.1% of the total). On normal skin sections, we observed 0 to 3 γ/δ+ cells per section. When present, they were often located in the epidermal basal layer, and were round or dendritic. Double immunolabeling revealed that γ/δ+ cells differed from CD1+ Langerhans cells, and that they had a similar phenotypic pattern as γ/δ+ peripheral lymphocytes (PBL): CD2+, CD3+, CD4−, and CD8−. Immunostaining from various inflammatory skin lesions showed that the dermal infiltrates included CD3+ T lymphocytes but virtually no γ/δ+ cells. Only a few γ/δ+ cells were found in some end-evolutive infiltrates. Taken together, these results strongly suggest that normal human epidermis occasionally harbors TCR-γ/δ complex bearing lymphocytes, which constitute a small fraction of the CD3+ cutaneous T lymphocytes. Despite their common location and TCR expression, there is presently no evidence that these human cutaneous TCR-γ/δ bearing T cells are the equivalent of murine DETC. *J Invest Dermatol 94:764–768, 1990*

Mammalian epidermis harbors ontogenically and functionally heterogeneous cell lines. One of the anatomically restricted cell populations inside the murine epidermis is composed of the previously so-called Thy-1+ dendritic epidermal cells [1,2], which are bone-marrow derived and distinct from Langerhans cells [3]. These cells were recently renamed dendritic epidermal T cells (DETC) [4] because they display the following surface T-lymphocyte phenotype: Thy-1+, asialo-GM1+, T3+, Lyt-1−, Lyt-2−, L3T4−, Ia−, and sIg− [1,2,5]. The human equivalent of DETC has not been visualized so far [6]. It was recently demonstrated that DETC express the T3-associated γ/δ heterodimer of the surface T-cell receptor (TCR) [5,7], which was recently characterized as another form of TCR in addition to the TCR-α/β heterodimer (for review, see [8]).

The first murine anti-human TCR-γ/δ monoclonal antibody (MoAb), namely, anti-TiγA, was developed against the Vγ9 gene product present on 70% of TCR-γ chain bearing peripheral blood lymphocytes (PBL) of most normal individuals [9]. Other MoAb subsequently became available, and react with either constant or variable regions of the TCR-δ chain. The present study was designed to determine whether T cells bearing the TCR-γ/δ complex are present in human epidermis, using the above MoAb.

MATERIALS AND METHODS

Human Skin Fresh epidermal sheets from the thigh were obtained from suction blister roofs, in five healthy volunteers. After washing in Mac Keehan's solution, the sheets were fixed in acetone for 10 min, and dried for 10 min before immunolabeling. Epidermal cell suspensions were also obtained after incubation for 15 min at 37°C with a 0.1% trypsin solution (Boeringher, Mannheim, West Germany). Spun smears from epidermal cell suspensions were air dried, fixed in acetone for 10 min, and stored in the same conditions.

In addition, we studied 26 non-lesional skin forearm biopsies, 44 lesional skin specimens (Table I), and five biopsies including infiltrates at the center or periphery of cutaneous lesions (vasculitis, seborrheic dermatitis, psoriasis, lupus-like lesion) from AIDS patients. These biopsies were snap-frozen in liquid nitrogen, freshly cut into 5-μm sections, air dried for 2 h, fixed in acetone for 10 min, and stored at −20°C until use.

Reagents The murine MoAb used, and their characteristics, are listed in Table II. The experiments using these MoAb were performed in successive stages, as the MoAb became available. The

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Abbreviations:
APAAP: alkaline-phosphatase–anti-alkaline-phosphatase complexes
DETC: Thy-1+ dendritic epidermal T cell
IEL: intra-epithelial lymphocyte
IF: immunofluorescence
MoAb: monoclonal antibody
PAP: peroxidase-anti-peroxidase complexes
PBL: peripheral blood lymphocyte
TCR: T-cell receptor

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Table I. Panel of Frozen Sections of Lesional Skin, Studied by Immunolabeling with CD3, Anti-TiγA, and Anti-TCR δ1 MoAb[a]

Inflammatory non-specific reactions	5
Atopic dematitis	3
Lichen planus	4
Lymphocytoma cutis	1
Jessner-Kanoff reaction	2
Discoid lupus	3
Subacute lupus	3
Drug eruption	3
Insect bite reaction	2
Psoriasis	3
Vasculitis	4
Granuloma	2
Scleroderma	2
Graft-versus-host disease	2
Bullous pemphigoid	2
Parapsoriasis	2
Mycosis fungoidis	1
Total	44

[a]Diagnoses were made by standard histologic examination.

rabbit asialo-GM1 antiserum (Wako Chemicals, Neuss, West Germany), raised against a glycolipid mainly expressed on murine DETC and NK cells, was also used as primary antibody. Rabbit anti-mouse Ig, swine anti-rabbit Ig, preformed calf intestinal phosphatase alkaline-mouse anti-phosphatase alkaline MoAb (APAAP) complexes, and peroxidase-rabbit anti-peroxidase MoAb (PAP) complexes were obtained from Dakopatts Ltd. (Glostrup, Denmark). Fast red TR salt (No F-1500), naphtol AS-TR phosphate (N-6000), fast blue BB salt (F-3378), naphtol AS-MX phosphate (N-4875), 4-chloro-1-naphtol (C-8890), and Levamisole were all purchased from Sigma (St. Louis, MO). Tris buffered saline (TBS) was prepared by adding Tris-HC1 buffer (0.5 M) to normal saline (0.15 M).

Immunolabeling Procedures After incubation for 10 min with 0.05 M TBS, pH 7.6, acetone-fixed epidermal sheets or spun epidermal cell smears, and frozen skin sections were respectively treated overnight at 4°C, and for 1 h at room temperature with appropriate dilutions of MoAb (Table II). Indirect immunofluorescence (IF) and APAAP procedures were then performed as previously described [1,10]. For APAAP, specimens were washed 3 times in TBS (0.05 M, pH 7.6), and treated for 1 h with rabbit anti-mouse Ig diluted 1:50 in TBS. After washing again, they were incubated with the 1:50 diluted APAAP complexes for 1 h, and washed in TBS (0.1 M, pH 8.2). The alkaline phosphatase substrate yielding a red reaction product was prepared by dissolving 20 mg naphtol AS-TR phosphate in 2 ml dimethylformamide, and then adding 100 ml TBS (0.1 M, pH 8.2) containing 100 mg fast red TR and 130 μl Levamisole (240 mg/ml). This mixture was filtered and applied onto the slides for 15–20 min. Finally, the specimens were rinsed in TBS and placed on glass slides in aquamount. The slides were counterstained by Mayer's hemalun. Frozen normal spleen was used as positive control.

Double immunolabeling on frozen skin sections were performed as described by Wagner and Worman [11]. This technique consisted of applying first anti-TCR δ1 or anti-TiγA MoAb onto the specimens and developing the APAAP procedure as above. The second APAAP labeling was subsequently performed on the same specimens with one of the following as second MoAb (used twice more concentrated): CD1, CD2, CD3, CD4, or CD8. In this part of the experiment, 10 mg fast blue BB and naphtol AS-MX phosphate was used in place of fast red TR and naphtol AS-TR phosphate, respectively, in order to provide a reaction product which contrasted with the first reaction product. To prevent any reaction between the second set of immunoreagents with unused binding sites from the first set, rabbit anti-mouse Ig and APAAP complexes of the first set were used more concentrated in order to saturate all the binding sites, i.e., 1:20 and 1:25, respectively. The color mixture due to remaining alkaline phosphatase activity after the first chromogene reaction was minimized by developing the second reaction for only 5–10 min under microscopic observation. Thus, controls could verify that: 1) the second labeling system showed the same proportion of positive cells in the sequential procedure as in the single APAAP; 2) a strong red-blue contrast between nonoverlapped cell types could be defined (for instance, CD1+ and CD3+ cells); and 3) similar labelings were observed by reversing the order of the MoAb-APAAP deposits. A APAAP-PAP double immunolabeling method was also performed as already described [12], using anti-TCR δl as first MoAb, and asialo-GM1 antiserum as second antibody. After incubation of this latter and washings, the swine anti-rabbit Ig diluted 1:40 in TBS, and the PAP complexes were subsequently applied, for 1 h each. The peroxidase substrate yielding a black reaction was obtained by adding for 5–10 min in the dark the following preparation: 20 mg 4-chloro-1-naphtol; absolute ethanol, 0.25 ml; 100 ml 0.05 M TBS, pH 8.2; and finally 10 μl H_2O_2.

RESULTS

Exposure of human epidermal sheets and smears of spun epidermal cells to MoAb against T-cell membrane antigens showed that CD3+ T cells were consistently found. These cells formed 0.5 to 1% of the total epidermal cells, as counted on smears (10^3 cells/smear). On frozen skin section from 26 normal individuals, the results also showed the presence of CD3+ cells, either interspersed regularly or more or less clustered along the basal layer of the epidermis (Fig 1), as well as CD3+ cells clustered around the vessels of the dermis. These cells seemed to be mostly TCR-α/β+, and either CD4+ or CD8+.

Using the panel of anti-TCR+γ/δ MoAb (Table II), very rare TCR-γ/δ+ cells were visualized forming less than 0.1% of the epidermal cells on sheets and smears, and 0–3 positive cells per section (Fig 2). Anti-TCR δ1 and BB3 MoAb revealed positive

Table II. MoAb Used in the Study

MoAb	Origin	Dilution	Antigen Specificity	Cell Specificity
Anti-TiγA	Dr. Hercend	1/200 (ascitis)	TCR-Vγ 9/JP	70–75% TCR-γ/δ + PBL (6% of total PBL)
Anti-TCRδ1	T-Cell Sciences	1/200	TCR-Cδ	± 100% TCR-γ/δ + PBL (± 8% of total PBL)
BB3	Dr. Moretta	1/100	TCR-Vδ AB12 ?	Disulfide-linked TCR-γ/δ + PBL (95% TiγA + PBL)
Anti-δTCS1	T-Cell Sciences	1/20	TCR-VIDP2/Jδ ?	Non-disulfide-linked TCR-γ/δ + PBL (± 1% of total PBL)
βF1	T-Cell Sciences	1/50	TCR-α/β	± 100% TCR-α/β + PBL
Leu 5	Becton-Dickinson	1/100	CD2	E-rosetted T cells
Leu 4	Becton-Dickinson	1/100	CD3	Mature T cells
IOT4	Immunotech	1/50	CD4	Helper T cells
IOT8	Immunotech	1/60	CD8	Cytotoxic/suppressor T cells
BL6	Immunotech	1/40	CD1	Langerhans cells/corticothymocytes
HML-1	Dr. Labrousse	1/400 (ascitis)	unknown	Intraepithelial lymphocytes

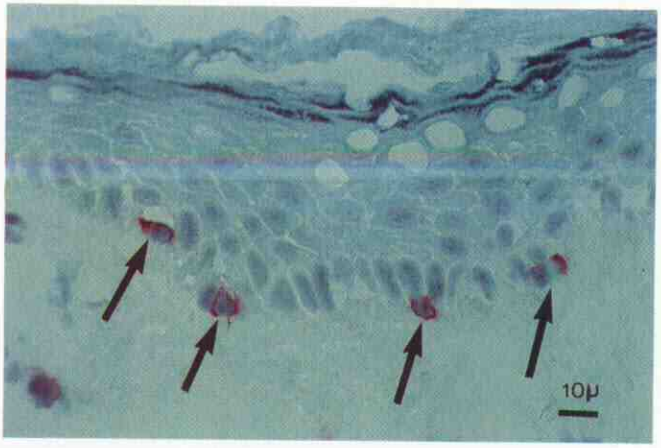

Figure 1. CD3+ T cells *(arrows)* demonstrated on normal skin sections by APAAP deposits. In the epidermis, these cells were often scattered among the basal keratinocytes, as shown here.

quantitative and morphologic patterns similar to those revealed by anti-Tiγ A MoAb. Many of these positive cells were round or oval. Dendritic cells were also observed. On sections, they were located in the basal and suprabasal layers of the epidermis and rarely in the upper dermis. No expansion in cell density was observed between individuals. With anti-δTCS1 MoAb, whose epitope is unfrequently associated with the Vγ9 anti-Tiγ A epitope in the blood γ/δ+ cells, virtually no positive cells were observed. Furthermore, unlike normal intestine epithelial used as control, no positive cells were stained on skin sections with HML-1 MoAb.

Double immunolabeling on skin sections revealed that TCR-γ/δ+ cells of the epidermis were CD1−, because they did not react with BL-6 MoAb. Furthermore, these cells seemed to display the same phenotypic characters as those defined for their counterparts in the peripheral blood [13], because they were also CD2+, CD3+ (Fig 3), CD4−, and CD8−. Unlike murine DETC, they were also asialo-GM1−.

To determine whether cutaneous TCR-γ/δ+ cells are involved in skin lesional conditions, 44 inflammatory skin infiltrates including lymphocytes (Table 1) were studied with CD3, anti-Tiγ A, and anti-TCR δ1 MoAb. In all the infiltrates, the proportion of CD3+ T lymphocytes varied greatly, from 25 to 70%. Most of the infiltrates studied lacked TCR-γ/δ+ cells. Only the infiltrates of three of five non-specific inflammatory reactions, and one of three discoid lupus reactions, were scattered with TCR-γ/δ+ cells (Fig 4). These cells comprised 2–5% of these infiltrates. Some positive cells migrated onto the epidermis facing the TCR-γ/δ+ infiltrates. We noted that these infiltrates were associated with the presence of histologic features evoking terminal reactions (e.g., absence of oedema and polymorphic nuclear cells, fibrotic thickening of the papillary dermis, and thickened vessel walls). However, five infiltrates including no TCR-γ/δ+ cell could also be classified as resulting from terminal reactions, according to the histologic features indicated above. Therefore, the presence of TCR-γ/δ+ cells in cutaneous infiltrates seems not specific to terminal reactions.

We further addressed the question whether, as suggested by the above results, TCR-γ/δ+ cells are involved in cutaneous reactions from suppressive conditions such as AIDS. Accordingly, we examined the lymphocytic infiltrates from various dermatoses (see *Materials and Methods*) in five AIDS-patient frozen biopsies. CD1, CD3, CD4, anti-Tiγ A, and anti-TCR δ1 were used as primary MoAb. On these sections, the number and dendritic morphology of CD1+ Langerhans cells were markedly reduced. CD3+ cells were distributed throughout the dermis and the epidermis, representing 10–30% of the total infiltrating cells, and CD4+ cells were virtually absent. Among these AIDS skin infiltrates, only 0–1 TCR-γ/δ+ cell/section was seen in the dermis or epidermis.

DISCUSSION

In a search for the genes encoding the α and β chains of the TCR complex, Saito et al recently discovered an additional class of rearranging TCR genes [14]. This discovery led to the characterization

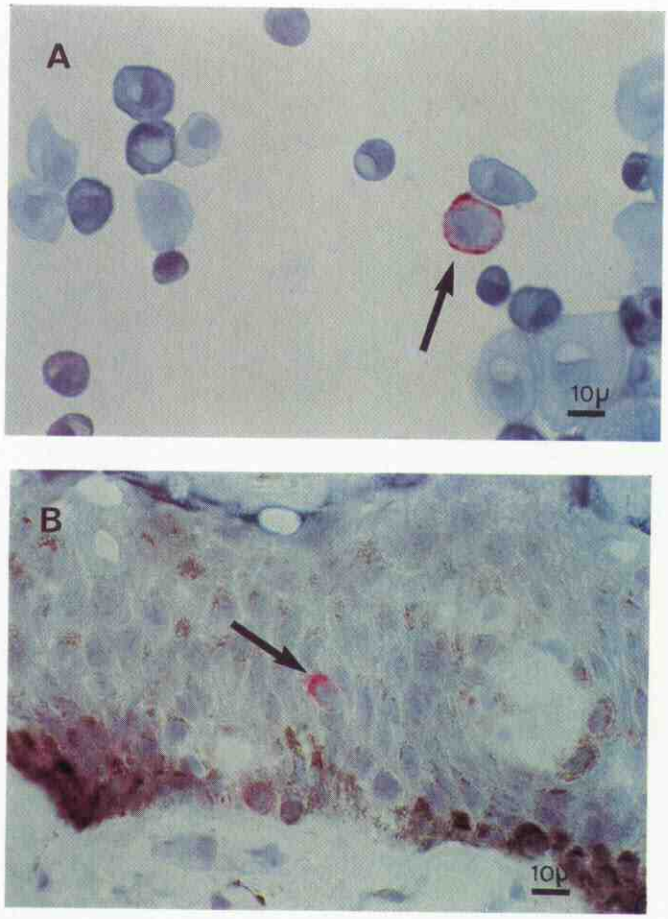

Figure 2. Identification of Tiγ A+ cells *(arrows)* using an APAAP technique on *(A)* spun EC smear (around 10^3 cells/smear), and *(B)* skin section from one normal individual.

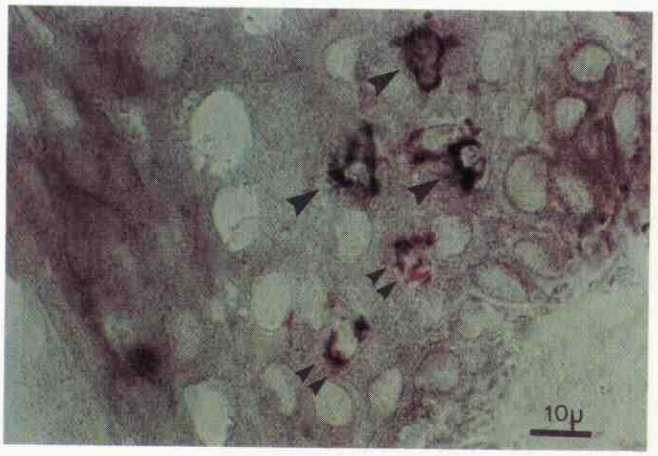

Figure 3. Double immunolabeling on forearm skin section from one patient. Here, red deposits reveal the Tiγ A+ cells *(double arrowheads)*, and blue-black deposits show the CD3+ cells *(single arrowheads)*. Note that some blue CD3+ T lymphocytes do not express the TCR-γ chain, whereas all the red Tiγ A+ epidermal cells express also the T-cell CD3 epitope.

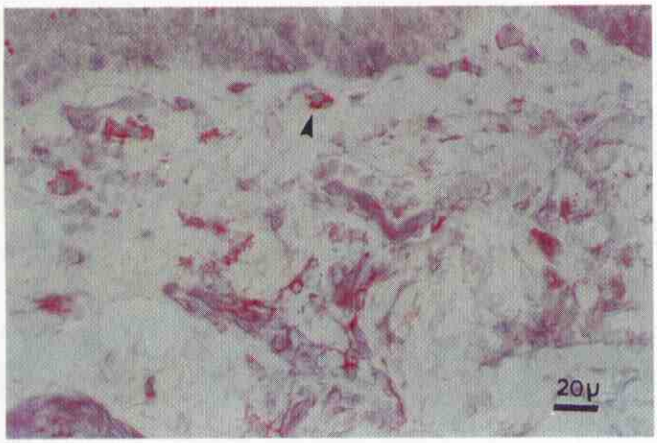

Figure 4. APAAP labeling of TiγA+ cells *(arrowhead)* within the dermal infiltrate of a non-specific inflammatory reaction.

of the γ/δ heterodimer of the TCR [15]. Using anti-TiγA, the first MoAb available against this heterodimer, Jitsukawa et al delineated the PBL subset that expresses this TCR-γ/δ complex, and showed that anti-TiγA MoAb reacted with all cloned cell lines expressing the variable V9/JP rearrangement of the γ chain [9]. Other MoAb which were developed later recognized a constant portion (anti-TCR δ1 MoAb) and variable portions (anti-δTCS1 and BB3) of the δ chain. The TiγA+ cell fraction was found to represent about 5% of the total PBL, although the inter-individual range varied from 1 to 10%, and the whole TCR-γ/δ+ fraction delineated by the anti-TCR δ1 MoAb comprised about 8% of the PBL (range, 1 to 15%) [13,16,17]. Virtually all CD3+, γ/δ+ PBL had the following phenotype: CD7+, CD2+, CD5+, CD1−, CD4−, CD16−, CD14−, CD19−, CD25−, and MHC class II−, but were heterogeneous with respect to their expression of CD8, CD11b, and CD28 [16,17]. On the other hand, it has been recently reported that most intra-epithelial lymphocytes (IEL) express the TCR-γ/δ receptor [18]. Taken together with the previous observation that DETC in murine epidermis bears exclusively γ/δ receptors [5], this might suggest that TCR-γ/δ bearing lymphocytes are anatomically restricted to epithelial tissues [19]. TCR-γ/δ+ cells appear to be true immunocompetent T cells. Indeed, it has been shown using functional assays that clones of murine DETC as well as human IEL and TCR-γ/δ+ PBL display NK-like activity [9,20,21]. Some of these cell lines are IL-2-dependent [21–23]. More precise functions have not been yet established, because the ligand bound by the γ/δ complex is still unknown.

The present study was designed to assess the distribution and the TCR phenotype of T cells in normal and lesional human skin. As demonstrated by Groh and co-workers [16,24], we confirmed that human epidermis and dermis normally harbor a few mature CD3+ T lymphocytes. These cells are predominantly α/β+ and either CD4+, or CD8+. On the other hand, a minority of epidermal T cells has been reported to have a CD3+, α/β+, CD4−, and CD8− phenotype [24]. Among the CD3+ lymphocyte population, we observed the presence of TCR-γ/δ+ cells. Using a series of specific MoAb allowing recognition of most of the cells, we showed that they constitute a small cell fraction of CD3+ epidermal T lymphocytes. We were not able to demonstrate, in normal skin, either large cell density variations like those in the peripheral blood [9,16], or elective location as in the intestinal epithelium [18]. It is clear that our TCR-γ/δ+ cells are distinct from Langerhans cells, because double marker studies revealed that most γ/δ+ cells were CD1−. We cannot, however, totally exclude the possibility that a minority of these cells display also the CD1 phenotype because of the small numbers of cutaneous γ/δ+ cells, or that they bear low levels of CD1 antigen. Furthermore, cutaneous γ/δ+ cells displayed the same phenotypic characters as TCR-γ/δ+ PBL, namely, CD2+, CD3+, CD4−, and CD8−, and a different phenotypic character from DETC, namely, asialo-GM1−. In our study, TCR γ/δ+ cells were only found in some terminal cutaneous reactions, and not in active lesional conditions. However, it is more likely that these findings are circumstantial than the evidence of a down-regulating cell signal, because TiγA+ cells did not infiltrate all the chronic terminal reactions studied. Furthermore, there was no counterbalance quantity in the skin of AIDS patients between TCR-γ/δ+ cells which again were very few, and CD1+ Langerhans cells or CD4+ helper T-cells, whose numbers were greatly reduced. Taken together, we could not demonstrate a specific expansion of human TCR-γ/δ+ cells in cutaneous conditions.

γ/δ+ IEL differs from γ/δ+ PBL by their prevalence over α/β+ IEL and their large expression of surface CD8 antigen [17,18]. DETC migrate ontegenetically very early after birth within murine epidermis and express an homogenous γ/δ gene rearrangement product [4]. These observations led to postulate that the γ/δ receptor is predominantly expressed by epithelial lymphocytes and influenced by epithelial factors [25]. In contrast, we found that TCR-γ/δ bearing lymphocytes of the human skin are similar by their proportion and phenotype to their γ/δ+ peripheral blood counterparts, that is, with a very minor γ/δ+ cell component. The recent report by Groh confirms the broaden repertoire of these cells at all immunologic relevant sites, without obvious tropism for epithelial tissue [16]. Therefore, human skin is not a prevalent tissue resident for TCR-γ/δ+ cells, unlike murine epidermis. Further insight into the phenotype and function of human TCR-γ/δ+ epidermal cells and murine DETC is necessary to clarify their respective lineages. In conclusion, although the functions of TCR-γ/δ+ epidermal cells are unknown, the presence of these cells opens up a new field of research on the immunologic role of the epidermis.

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SYMPOSIUM ON HEREDITARY DISORDERS OF KERATINIZATION

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